New treatment options in the management of IBD – focus on colony stimulating factors

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Abstract: Inflammatory bowel disease (IBD) is characterized by inflammation of the gastrointestinal tract, typically with a relapsing and remitting clinical course. The intestinal inflammation in IBD is controlled by a complex interplay of innate and adaptive immune mechanisms. Innate immunity comprises a set of distinct elements, which includes circulating cells such as neutrophils, monocytes, and resident intestinal immune cells (dendritic and Paneth cells), as well as intestinal epithelium and cellular products, including antimicrobial peptides such as defensins and cathelicidins. Different components of innate immunity in IBD have been suggested to be defective or impaired. The human granulocyte-macrophage colony-stimulating factor (GM-CSF) and the human granulocyte colony-stimulating factor (G-CSF) have emerged as potential tools for the modulation of intestinal inflammation and repair. The greatest evidence supporting the use of colony-stimulating factors in intestinal inflammation comes from studies conducted in active Crohn's disease (CD) patients treated with sargramostim and filgrastim, but evidence for its recommendation as treatment remains weak, as the majority of studies are open label, nonrandomized, and with a small number of patients.

Keywords: inflammatory bowel disease, colony stimulating factors, sargramostim, filgrastim

Introduction

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), represents a group of chronic disorders characterized by inflammation of the gastrointestinal tract, typically with a relapsing and remitting clinical course. Mucosal macrophages play an important role in the mucosal immune system, and an increase in the number of newly recruited monocytes and activated macrophages has been noted in the inflamed gut of patients with IBD. Activated macrophages are thought to be major producers of inflammatory cytokines in the gut, and imbalance of cytokines contributes to the pathogenesis of IBD. The intestinal inflammation in IBD is controlled by a complex interplay of innate and adaptive immune mechanisms, where colony-stimulating factors (CSF) may have a potential therapeutic role that remains to be strongly proved.

The potential usefulness of CSF in IBD emerged from the knowledge of the underlying immunological disorders in genetic diseases associated with CD or with a Crohn's-like intestinal disease, such as chronic granulomatous disease (Myrup et al 1998), glycogen storage disease Ib (Roe et al 1992), and cyclic neutropenia (Fata et al 1997), among others, where CSF had been shown to be useful. At this time, there is evidence that CSF may be an alternative treatment for IBD, but evidence is still insufficient to recommend it in clinical practice.

Innate immunity in IBD

Innate immunity comprises a set of distinct elements, which includes circulating cells such as neutrophils, monocytes, and resident intestinal immune cells (dendritic and

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Paneth cells) as well as intestinal epithelium and cellular products (defensins and cathelicidins). Different components of innate immunity in IBD have been suggested to be defective or impaired (Yamamoto-Furusho and Korzenik 2006), and there remain the potential therapeutic effects of CSF.

Below we describe the sites where CSF may act in the pathogenesis of IBD related to immune defects.

Neutrophil defects and impaired mucosal innate immune response

Several defects have been described in patients with CD, including impairment in migration of neutrophils (Segal et al 1976); complement dysfunction that produces impair neutrophil recruitment (Elmgreen 1986); decreased phagocytic and bactericidal neutrophil functions (Wandall 1985); and deficient superoxide generation in neutrophils (Curran et al 1991).

A significantly diminished production of IL-8 and IL-1β (45% and 50% reduction, respectively) was found from macrophages of patients with CD (Marks et al 2006). Despite previous emphasis on adaptive immune dysfunction, there is evidence that patients with CD possess a weak acute innate inflammatory response (Rahman et al 2008). This failure of inflammatory mediator production leads to insufficient recruitment of neutrophils, resulting in inadequate removal of bacteria and other debris (Marks and Segal 2008), perpetuating the aberrant inflammatory response. The increased tissue migration of neutrophils with CSF therapy may explain its potential benefits in CD (Harbord et al 2006).

Effect of NOD2 mutations

NOD2 is a cytoplasmic protein that serves as a microbial sensor, and its leucine-rich repeat (LRR) domain is required for recognition of muramyl dipeptide (MDP), a fragment of peptidoglycan present in bacterial cell walls. Membrane recruitment of NOD2 is essential for NF-kB activation after the recognition of MDP and exerts antibacterial activity in intestinal epithelial cells.

Specific mutations of the NOD2 gene (Arg702Trp, Gly908Arg, and leu1007fsinsC) produce selective functional defects in leukocytes of patients with CD as shown by van Heel et al (2005) who analyzed cytokine expression of peripheral blood mononuclear cells after exposure to muramyl dipeptide (MDP). In PBMC from CD patients the NOD2 ligand induced little TNFα and IL-1β, but strong IL-8 secretion. Furthermore, monocytes isolated from CD patients carrying the 1007fs (3020insC) mutation were reported to exhibit defects in the production of the proinflammatory cytokines, TNFα, IL-6 and IL-8, as well as the anti-inflammatory cytokine IL-10 (Netea et al 2005). These findings were recently confirmed by Beynon et al (2008), who also found an impaired release of IL-12p40, which may be a new link between NOD2 mutations and the inflammatory mechanisms. Dendritic cells derived from CD patients homozygous for leu1007fsinsC also fail to up-regulate the costimulatory molecules CD80 and CD86 in response to MDP and lack production of cytokines such as TNF-α, IL-12, and IL-10 (Kramer et al 2006).

In general, α-defensins (1–3, 5, and 6) production by Paneth cells is diminished in the colonic mucosa of CD and UC patients. NOD2 mutations in CD patients have been associated with diminished mucosal α-defensin expression (Wehkamp et al 2004), but recently it was found that this reduction could be independent (Simms et al 2008). Decreased β-defensin 1 and the lack of induction of inducible antimicrobial peptides β-defensins 2 and 3 in CD could result in enhanced bacterial survival and perhaps invasion (Fellermann et al 2003), probably leading to activation of the previously explained impaired immune response and triggering IBD.

Experimental and preclinical studies

The human granulocyte-macrophage colony-stimulating factor (GM-CSF) was shown to be clinically and histologically effective in dextran sulfate sodium-induced acute colitis model in mice (Sainathan et al 2008).

Immune complex colitis was induced in White New Zealand rabbits to evaluate the effect of the human granulocyte colony-stimulating factor G-CSF 50–200 μg/kg on colonic mucosal inflammation, neutrophil recruitment, and the generation of eicosanoids. It was found that therapy with G-CSF resulted in a marked decrease of proinflammatory mediators, but mucosal generation of the protective prostaglandin E2 was preserved, suggesting that it may have anti-inflammatory effects in colitis (Hommes et al 1996). G-CSF also showed an effect on preventing the onset of Th1-type 2,3,6-trinitrobenzene sulfonic acid-induced (TNBS) colitis in mice (Yoshimitsu et al 2006).

Polymorphonuclear neutrophils apoptosis in tissues of colonic mucosa of UC and CD patients may be delayed under the influence of G-CSF (Kenji et al 1999). Furthermore, G-CSF therapy increases neutrophil tissue migration in CD, which may be involved in its therapeutic effect (Harbord et al 2006).

Options for colony-stimulating factors

Growth factors have recently emerged as potential tools for the modulation of intestinal inflammation and repair.

These factors play an important role in the modulation of cellular proliferation, differentiation, angiogenesis, and inflammation. At least 30 different growth factors are relevant for the maintenance of gut mucosal integrity, including GM-CSF and G-CSF. The greatest evidence supporting the use of colony-stimulating factors in intestinal inflammation comes from studies conducted in patients with active CD: sargramostim and filgrastim.

GM-CSF

Initial findings suggested that patients treated with sargramostim (GM-CSF) (Immunex Corporation, Seattle, WA, USA) in active CD had a high rate of clinical response and remission, with limited side effects (Dieckgraefe and Korzenik 2002). This 8-week, open-label, dose-escalating study, was conduced on 15 patients with Crohn's Disease Activity Index (CDAI; Best et al 1976) greater than 220 but lower than 475. Among them, 80% achieved clinical response (decrease in CDAI $<$ 70 points) and 53% achieved remission (CDAI $<$ 150). The response rate was 75%, 85%, and 75% in the 4, 6, and 8 μg/kg/day dose groups. The only patient with a fistula had complete clinical closure of a chronic rectovaginal fistula. Treatment with sargramostim also improved quality of life.

Lastly, the Sargramostim in Crohn's Disease Study Group (Korzenik et al 2005) conducted a multicenter, randomized, placebo-controlled trial, which included 124 patients. Patients were randomly assigned in a 2:1 ratio to receive sargramostim (6 μg/kg body weight) or placebo subcutaneously daily for 56 days. The primary end-point was clinical response and other end-points included changes in disease severity, quality of life, and adverse events. Of the 81 patients in the sargramostim group, 57 (86%) completed treatment, and of the 43 of the placebo group, 37 (86%) completed treatment.

The primary end-point was not proven; this was achieved in 54% in the sargramostim group and 44% in the placebo group ($p = 0.28$), but a clinical response defined by a decrease from baseline of at least 100 points in the CDAI score was significantly higher in the sargramostim group than in the placebo group (48% vs 26%, $p = 0.01$), as well as the remission rate (40% vs 19%, $p = 0.01$). The improvement, including remission rates, was also superior in the sargramostim group 30 days after treatment. In patients in whom the validated Crohn's Disease Endoscopic Index of Severity was assessed, the median post-treatment scores were significantly lower in the sargramostim group, but the median decrease between the two groups was not. Draining fistulae was eliminated in 4 of 8 of patients in the treatment group and in 2 of 5 in the placebo group. Only 1 of 78 patients had detectable neutralizing antibodies at day 57, and no association was observed with adverse events.

Treatment with GM-CSF may provide effective synergistic or single-agent treatment alternatives to immunosuppression for IBD (Dieckgraefe et al 2006), but evidence that supports its recommendation as treatment remains weak.

G-CSF

Dejaco et al (2003) performed an open-label pilot study with filgrastim (Neupogen®, Amgen Inc, Thousand Oaks, CA, USA) in 5 CD patients with severe endoscopic postoperative recurrence, but with clinically inactive CD (CDAI < 150). Patients received 300 μg of filgrastim subcutaneously, 3 times weekly for a total of 12 weeks, for the primary objective of evaluating safety and efficacy in this group of patients. Efficacy was evaluated by ileocolonoscopy, including histopathological examination according to Rutgeerts' rating for postoperative recurrence (Rutgeerts et al 1990), which was performed before treatment and within 1 week after the end of treatment. Four patients had stricturing and 1 had penetrating behavior. Complete mucosal healing occurred in 2 patients after treatment (1 patient after 12 weeks of therapy and in 1 patient 9 months after treatment cessation), and all other patients had no response. In 1 patient closure of perianal fistulas was noted. This study suggested that despite the small number of patients, filgrastim seems to be safe, well tolerated, and might provide efficacy in CD.

Korzenik and Dieckgraefe (2005) conducted a 12-week open-label trial with filgrastim (Neupogen®, Amgen Inc, Thousand Oaks, CA, USA) that offered preliminary evidence that it is a safe and potentially effective therapy for the treatment of active CD and fistulous complications. Twenty CD patients with a CDAI $>$ 220 and $\leq$ 450 were enrolled. Primary end-point was a decrease in the CDAI of $>$ 70 points and remission was considered to be a CDAI $<$ 150 points. All patients received filgrastim daily for 12 weeks at an initial dose of 300 μg subcutaneously. The absolute neutrophil count (ANC) was monitored weekly and was targeted between 25 and 35×10^9. The dose was adjusted downward by 100 μg if ANC exceeded this range, and after a subsequent reduction to 100 μg/day, the dose was lowered to 75 μg/day. Five patients (25%) achieved remission during the study, 11 (55%) demonstrated a decrease of at least 70 points, and 3 of 4 (75%) patients with fistulae had a positive response (defined as closure of more than 50% of fistulae). Among responders at week 12, 4 of 11 patients (36%) maintained

response for additional 4 weeks after completion of therapy and the others had an increase in disease activity.

Conclusions

Cellular or animal models, and in vivo experiments support the hypothesis that CD may result from innate immune deficiency characterized by an aberrant innate immune response occurring more proximately, leading to T cell activation. Several lines of evidence such as functional implications of NOD2 mutations, and observations of CD-like manifestations, have converged to present a coherent hypothesis that impaired innate immunity initiates the cascade of events resulting in CD. GM-CSF, a more potent stimulator of innate immune function than G-CSF, seems to have a beneficial effect in patients with active CD. Finally, as the majority of studies are open label, nonrandomized, and with a small number of patients, there is no strong evidence of the effect of GM-CSF and G-CSF as treatment for IBD, so evidence to supports its recommendation as a treatment is still weak.

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Disclosures

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